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## METHOD FOR SOLUBILIZING INSOLUBLE COLLAGEN FIBERS

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9 Claims. (Cl. 195-6)

This is a continuation-in-part of my copending patent application Ser. No. 365,197 filed May 5, 1964 and now abandoned.

The present invention relates to a method for solubilizing insoluble collagen. Collagen, as is well known, is the principal proteinaceous constituent of skins of all types. The present invention is concerned particularly with the insoluble collagens occurring in animal hides.

If fresh native collagen is immersed in aqueous solutions of salts, such as sodium chloride, or acids, such as hydrochloric acid, some portion thereof will dissolve. However, significant portions of the collagen will remain undissolved. From a commercial standpoint, it is desirable to have a method for solubilizing all of this collagen in such a form that it can be reconstituted in a fiber resembling native collagen.

The molecular structure of collagen has not been fully ascertained. It is believed that the molecular structure may be considered in a simplified form (for the purpose of this invention) as composed of two portions. One portion, the main part of the collagen molecule, is a macromolecule of a rigid rod-like character and having a three-chain helical configuration. The length of this main portion is in the range of 2700 A. to 3000 A. The second portion of the collagen molecule may be designated as the "end linkage," or "telopeptide group." This telopeptide group joins macromolecules not covalently, but successively in an end-to-end manner, thereby forming a polymeric string. The telopeptide linkage also is proteinaceous but it is believed to lack the highly-ordered characteristic of the main macromolecule.

In native collagen fibers, the polymeric strings of collagen macromolecules are in a staggered aggregate with the telopeptide groups of each polymer string being aligned approximately one-fourth of the way along the main portions of the macromolecules of adjacent polymer strings. Thus, the staggered aggregate yields electron micrograph patterns having a characteristic period of about  $\frac{1}{4}$  of the length of the macromolecule, or between about 640 and 700 A.

The lateral inter-molecular forces holding together the aggregates of the macromolecular polymers of collagen have not been fully characterized. In general, it is believed that these forces involve side-chain interaction between adjacent macromolecules. In the case of the soluble collagens, the forces are of a sufficiently low strength that the macromolecule polymers may be dispersed in acid or salt solutions.

As is well known, native collagen is relatively immune from attack by most enzymes except under certain conditions. The sole significant exception is the enzyme collagenase which is capable of reducing collagen to polypeptides. However, denatured collagen (in which the chains of macromolecule have been randomly disordered) is subject to attack by the usual proteolytic enzymes. From these facts, it has been suggested that immunity to enzyme attack is conferred by the three-chain helical structure.

Studies on solutions of soluble collagen have indicated that the telopeptide groups extend beyond the helical macromolecule and, as mentioned, are believed to have a disordered structure. According to this theory, they

should be liable to attack by the usual proteolytic enzymes (e.g. those other than collagenase). The theory has been confirmed by demonstrating that proteolytic enzymes such as trypsin and pepsin will partially digest the telopeptide groups of soluble collagen. The enzyme also exhibits a depolymerizing action. Collagen can be precipitated from the resultant digest showing that the monomeric macromolecular collagen helix has been unaffected by the enzyme digestion.

Despite the discoveries that the telopeptide groups of soluble collagen could be partially digested, similar digestion of insoluble collagen was achieved only with difficulty. This was believed to be attributable to the structure thereof.

The three constituent polypeptide chains of collagen are not cross-linked at first after its biosynthesis. By the time of biological maturation of soluble collagen, two or three of these chains are cross-linked intra-molecularly and covalently at the telopeptide group. The insoluble collagen is further cross-linked. Thus, in the case of young calf-skin, two or more macromolecules of collagen are also inter-molecularly and covalently cross-linked at the telopeptide groups (thereby insolubilizing the soluble collagen having only intra-molecular cross-linking). With further biological aging (i.e. as the calf-skin becomes the steer hide of an adult animal) additional intra- and inter-molecular cross-linking takes place. It is believed that the enzyme digestion of the telopeptide groups of the insoluble collagen of steer hide is very slow (as compared with the analogous digestion of calf-skin) because of the additional cross-linking.

Some years ago it was found that under certain conditions insoluble collagen of calf-skin could be digested with the common proteolytic enzymes. More specifically, under acid conditions enzymes such as pepsin will attack the insoluble collagen of calf-skin and yield a clear solution. Native-type collagen fibers can be reconstituted from the digest. The enzyme digestion is analogous to the depolymerizing action of the usual proteolytic enzymes on the soluble collagens mentioned above.

At that time, it was believed that the solubilization of the native insoluble collagen of adult animals with the common proteolytic enzymes was difficult unless the collagen had been denatured. A method for achieving such digestion, however, is disclosed in my United States Patent No. 3,121,049, granted Feb. 11, 1964.

According to the present invention, a novel process for solubilizing insoluble collagens, especially the insoluble collagen of steer hide has now been discovered. This method results in a collagen solution in a single easy and rapid step from which collagen fibers resembling native collagen can be reconstituted by methods known in the art.

According to the present invention, it has been found that if the enzyme digestion is carried out in the presence of water soluble neutral salts of divalent metals, the salts having the property of solubilizing soluble nature collagen without enzyme treatment or in the presence of cationic surfactants, the telopeptide group is rendered liable to proteolytic attack, while at the same time the main portion of the collagen molecule retains its resistance to attack by the enzymes exhibiting activity with respect to the telopeptide group. Thereby it is possible to obtain a solubilized collagen which, by methods known in the art, is reconstitutable in the form of collagen fibers resembling native collagen.

Neutral salts which can be employed in the present invention are the water-soluble chlorides, sulfates or acetates of bivalent metals selected from the group consisting of calcium, magnesium, barium, strontium, zinc, cadmium and manganese. The foregoing salts may be used generally in concentrations between about 0.01 M and 1.5